THE MORPHOLOGY OF MOTILE AND ENCYSTED *ENDAMOEBA RANARUM* IN CULTURE

BY

W. H. TALIAFERRO

AND

ANNA B. FISHER

(*Department of Hygiene and Bacteriology, University of Chicago*)

(*Received for publication* 10 *November*, 1925)

PLATE VI

All of the amoebae used in this study were obtained from cultures started and furnished us by Barret and Smith. These investigators originally started their cultures from amoebae found in tadpoles and a detailed description of their technique will be found in their paper, immediately preceding this one. The present paper indicates beyond reasonable doubt that these authors have successfully cultivated an *Endamoeba* which, in culture, goes through the usual stages observed in the body, viz., active amoebae, precystic amoebae and cysts, and furthermore, that the specimens in all stages of development are identical with the description given by various authors of *Endamoeba ranarum* Grassi, 1879. In our previous study (Taliaferro and Holmes, 1924) of the morphology of an entozoic amoeba from the turtle, which Barret and Smith had cultivated, we deemed it necessary to study the forms from both the turtle and cultures. In the present case, however, where the form with which we are dealing has been studied by many investigators who have given excellent descriptions of it, it seemed necessary to consider only the amoebae from the cultures.

At the outset we wish to emphasize that a morphological study is always essential before an author is justified in concluding that he has actually cultivated an entozoic species. In fact, omissions of

this kind have been the basis of much confusion in the earlier attempts to cultivate entozoic amoebae. Thus, free-living contaminants have been mistaken for the entozoic species which were supposed to have been cultivated.

Since a review of the earlier work may be found in Dobell (1909) and much of the later work in Nöller (1922), no attempt will be made to review the various papers on the morphology of *E. ranarum*. Suffice it to say that Dobell has given an accurate description of the organism—a description which agrees in all major details with that given in the present paper. Moreover, not only can we compare the present cultural forms with the description by Dobell, but we can also make use of the resemblance between *E. ranarum* and *E. histolytica*. This will be most helpful, since the structure of *E. histolytica* has been so widely studied and is so well known. Dobell, in a footnote to the paper cited, calls attention to the extraordinary 'resemblance' between Hartmann's figures of *E. tetragena* (= *E. histolytica*) and isolated stages in *E. ranarum*. This remarkable resemblance between *E. histolytica* and the parasite of the frog has been noted by several subsequent workers. It even led Alexeieff (1914) to suggest that the harmless commensal of the frog, when introduced accidentally into man, might become the parasite of amoebic dysentery. The infection experiments of Dobell (1918) invalidate this conclusion, however, and indicate that the two species are distinct. With regard to the morphological similarity of the two, Dobell (1918) states, 'The active amoebae can usually be readily distinguished from one another by the inclusions (food bodies) in their protoplasm, but not by their own nuclear and cytoplasmic structure; but the precystic amoebae, devoid of all food bodies, and the cysts, at every stage of development, are so closely alike that preparations of the one could be used as demonstrations of the other.'

MATERIAL AND METHODS

As previously stated, all of our material was supplied us in culture by Barret and Smith. These cultures were subcultured on Barret and Smith's medium in this laboratory for several months. In the previous work of Taliaferro and Holmes (1924) on *Endamoeba barreti*

from the turtle, it was found that rabbit serum or Loeffler's dehydrated beef serum could be substituted for human serum in the original Barret medium. In our rather limited experience with *E. ranarum*, on the other hand, we have not obtained anything like as satisfactory results with rabbit or pig serum, Loeffler's dehydrated beef serum or human ascitic fluid, as we did with human serum.

All observations of living specimens were carried out at room temperature with material mounted under cover glasses sealed with vaseline. All of the prepared slides were fixed in Schaudinn's alcohol-sublimated mixture with 2 per cent. acetic acid. They were stained with Delafield's or Heidenhain's iron-haematoxylin with and without counter stains. No fixative was used to get the amoebae to adhere to the slide. As a consequence, the larger cysts tended to wash off the slides during the process of staining and dehydration (see differences in measurements of living and prepared cysts).

We have made a very careful study of the size of the present form in all three stages of its development, viz., active and precystic amoebae and cysts. In all cases the forms were drawn with a camera lucida, generally at a magnification of × 3,000, and the drawings measured. Owing to the characteristic circular shape of the precystic amoebae and the cysts, their size is given as their diameter in microns, but, since the active amoebae are generally very irregular in outline, their measure of size is given as the diameter of a circle having approximately the same area as the amoeba. (This method is similar to that used by Taliaferro and Holmes on *E. barreti*.)

With regard to size, active amoebae were measured from prepared slides ; (1) from a culture containing only active forms, i.e., containing no cysts ; (2) from a culture in which cysts had just begun to appear, and (3) from a month-old culture in which there was a large number of cysts. Their range in size varied as follows :—

No. measured	Range	Average
(1) 60	18·3μ to 38·0μ	26·3μ
(2) 53	12·0μ to 38·5μ	23·1μ
(3) 45	13·2μ to 26·0μ	19·0μ

The progressive decrease in average size which is brought out by these measurements is just what would be expected, since parasitic amoebae habitually grow smaller in preparation for encystment. In

passing, we may note that the size of these forms is within the range of size recorded for *E. ranarum* in the literature. Furthermore, the size of the large active amoebae (18·3μ to 38·0μ, average 26·3μ) is quite similar to the size of *E. histolytica*. Dobell (1919) gives the usual range of *E. histolytica* as 20μ to 30μ, and the extreme range as 18μ to 40μ.

The range in size of cysts studied in iodine was 9·6μ to 20·6μ, with an average of 14·8μ. Later, 105 cysts from the same culture, but drawn from prepared slides, showed a range of 6·3μ to 14·5μ, with an average of 10μ. In view of Dobell and Jepp's (1918) study of the size of *E. histolytica*, we might have expected a slight decrease in diameter (about 10 per cent.), but nothing like the one observed. We believe that the discrepancy is due simply to the fact that the smaller cysts adhere to the slide better than the larger ones. Indeed, this is borne out by the fact that in destaining the slides, one can actually see the large cysts being washed off. Therefore, we are probably justified in giving the range of size of the cysts in our cultures as from about 6μ to 20μ. The great similarity of these measurements to those of both *E. ranarum* and *E. histolytica*, is apparent when it is recalled that the diameter of cysts of *E. ranarum* is given as 10μ to 16μ by Nöller (1922) and of *E. histolytica* as 5μ to 20μ, by Dobell (1919).

The appearance of the active amoebae in the cultures depends largely on the food available. In the first cultures which contained flagellates, the endoplasm was generally so loaded with these organisms as to obscure the nucleus. In later cultures which were free of flagellates the motile amoeba appeared much like that shown in fig. 1. Specimens, for some time after being placed on a slide, assumed an elongated shape and progressed by means of lobose pseudopodia. These were extruded as masses of clear ectoplasm into which, later, the endoplasm flowed, and were formed from alternate sides of the anterior portion of the animal (fig. 1). When the slide began to dry up, the amoebae generally assumed a more spherical shape and extruded—almost explosively—clear hyaline pseudopodia without any evidence of active progression. In this condition they were almost identical in appearance with *E. histolytica* as seen in ordinary mounts of fresh faeces. The nucleus could be generally seen as a ring of dense material in which was embedded a

number of bright refractile granules (fig. 1). The karyosome was rarely visible in the living specimen although it was visible in the specimen from which fig. 1 was drawn.

The general appearance of the active amoebae in stained preparations is shown in fig 6, and the nuclei of two other specimens in figs. 7 and 8. The endoplasm of the specimen in fig. 6 was crowded with food vacuoles containing flagellates. The structure of the nucleus is interesting because it possesses all of the distinguishing characteristics of *E. histolytica*, such as the delicate layer of chromatin around the periphery, the small centrally-placed karyosome which is shown in figs. 7 and 8, and the linin network between the karyosome and periphery which is devoid of chromatin. In deeply-stained specimens, the karyosome cannot be seen (fig. 6)—most probably owing to its being obscured by the overstained linin network.

The large active amoebae frequently have more than one nucleus. In the sixty amoebae drawn to give the measurements discussed in a previous paragraph, six possessed two nuclei and one possessed four.

At no stage do any of the specimens ever show any trace of a contractile vacuole.

In cultures at the height of their growth there is little or no tendency for the amoebae to encyst, but as the cultures grow older the amoebae become smaller and more sluggish. In time these forms lose all food inclusions and become typical precystic amoebae. Fig. 9 shows a specimen which is probably intermediate between the large active form and the precystic form, whereas fig. 10 shows a typical precystic amoeba. These are even more like *E. histolytica* than the active forms. Their nuclei, as shown in fig. 10, are in every respect identical with that of *E. histolytica*, and in our experience, both the karyosome and achromatic capsule are more clearly seen than in the motile forms.

When the precystic forms encyst they are again identical with the cysts of *E. histolytica*. Figs. 2, 3 and 4 show their general appearance when alive. Quite frequently one or two nuclei can be made out in the living cysts, but it is rare to see all four, and sometimes none are visible. One nucleus is barely discernible in figs. 2 and 3, respectively. The chromatoid bodies appear rodlike and the glycogen masses as dull inclusions. The nuclei can be easily counted when the cysts are observed in iodine or stained with iron-

haematoxylin. Fig. 11 shows a cyst with one nucleus which is probably in an early stage of division, and figs. 12, 13 and 14 show mature quadrinucleate cysts. Each of these contain chromatoid bodies so diagrammatically like *E. histolytica* as to need no further description. Of the several hundred cysts examined from culture, none contained more than four nuclei, a condition once more similar to *E. histolytica*, for although there is some evidence that *E. histolytica* occasionally forms a supernucleate cyst with eight nuclei, the occurrence must be extremely uncommon. We have never encountered in our cultures any cysts suggesting the figures of Mercier and Mathis (1918), in which they depict their so-called " schizogonic " cysts.

SUMMARY

1. A detailed description is given of the amoebae from cultures originally isolated, by Barret and Smith, from tadpoles. The structure of these amoebae agrees in minutest detail with the descriptions by other authors of *Endamoeba ranarum* from the frog.

2. In culture, *E. ranarum* goes through the typical development of a parasitic amoeba, eventually forming cysts.

3. In agreement with all recent investigators, the present investigation emphasizes the similarity in structure of *E. ranarum* and *E. histolytica*.

REFERENCES

Alexeieff, A. (1914). Notes protistologiques. *Zool. Anzeiger*, Vol. XLIV, pp. 193-213.

Barret, H. P. (1921). A method for the cultivation of *Blastocystis*. *Ann. Trop. Med. & Parasitol.*, Vol. XV, pp. 113-116.

Barret, H. P., and Smith, N. M. (1924). The cultivation of an *Endamoeba* from the turtle, *Chelydra serpentina*. *Amer. Jl. Hyg.*, Vol. IV, pp. 155-169.

Boeck, W. C., and Drbohlav, J. (1925). The cultivation of *Endamoeba histolytica*. *Amer. Jl. Hyg.*, Vol. V, pp. 371-407.

Chiang, S. F. (1925). The rat as a possible carrier of the dysentery amoeba. *Proc. Nat. Acad. Sciences, U.S.A.*, Vol. XI, pp. 239-246.

Cutler, D. W. (1918). A method for the cultivation of *Entamoeba histolytica*. *Jl. Path. and Bact.*, Vol. XXII, pp. 22-27.

Dobell, C. (1909). Researches on the intestinal protozoa of frogs and toads. *Quart. Jl. Micro. Science*, N.S. Vol. LIII, pp. 201-277.

——— (1918). Are *Entamoeba histolytica* and *Entamoeba ranarum* the same species? *Parasitol.*, Vol. X, pp. 294-310.

——— (1919). *The amoebae living in man: a zoological monograph.* London.

Dobell, C., and Jepps, M. W. (1918). A study of the diverse races of *Entamoeba histolytica* distinguishable from one another by the dimensions of their cysts. *Parasitol.*, Vol. X, pp. 320-361.

Drbohlav, J. (1925a). Culture d'*Entamoeba gingivalis* (Gros, 1849), Brumpt, 1913. *Ann. Parasitol.*, Vol. III, pp. 361-363.

——— (1925b). Culture d'*Entamoeba coli* Loesch, 1875, emend Schaudin [n], 1903. *Ibid.* Vol. III, pp. 364-366.

——— (1925c). Culture d'*Entamoeba aulastomi* Nöller, 1919. *Ibid.* Vol. III, pp. 367-368.

Mercier, L., and Mathis, C. (1918). Kystes gamogoniques et schizogoniques chez *Entamoeba ranarum*. *Bull. Soc. Path. Exot.*, Vol. XI, pp. 47-54.

Nöller, W. (1922). *Die wichtigsten parasitischen Protozoen des Menschen und der Tiere.* I. Die parasitischen Rhizopoden. Berlin.

Taliaferro, W. H., and Holmes, F. O. (1924). *Endamoeba barreti*, n.sp., from the turtle, *Cheyldra serpentina*, etc. *Amer. Jl. Hyg.*, Vol. IV, pp. 160-168.

EXPLANATION OF PLATE VI

All figures are of *Endamoeba ranarum* from cultures in Barret's medium, reproduced at a magnification of × 1400. All drawings were made from camera lucida sketches, although that of the living motile amoeba (fig. 1) was necessarily largely a free-hand drawing. Stained specimens (figs. 6-14) were all fixed in Schaudinn's fluid and stained with Delafield's iron-haematoxylin without counter stain.

Fig. 1. Active amoeboid form as seen in living condition from a culture which had been freed of all other species of protozoa. The endoplasm contains a number of rod-like bacteria. Note that the karyosome of the nucleus is visible, although this is an exceptional occurrence in the living organisms.

Figs. 2, 3 and 4. Cysts from culture as seen in the living condition. Note that each cyst contains chromatoid bodies ; that figs. 3 and 4 contain one and two glycogen masses, respectively ; and that in figs. 2 and 3, a nucleus is visible.

Fig. 5. A cyst in iodine from the same culture as the one shown in fig. 2. Note the four nuclei. No chromatoids are visible but there is a rather lightly-stained glycogen mass indicated near the top of the cyst.

Fig. 6. Active amoeboid form fixed in Schaudinn's fluid and stained with Delafield's iron-haematoxylin. This form came from a culture which contained intestinal flagellates. The food vacuoles contain débris from the digestion of these flagellates. A definite karyosome is not seen in this specimen.

Figs. 7 and 8. Nuclei of active amoeboid forms fixed and stained in the same manner as the specimen shown in fig. 6. A karyosome is visible in each.

Fig. 9. An amoeboid form (fixed and stained as noted) which is probably intermediate between the large active amoebae and the precystic forms.

Fig. 10. A typical precystic amoeba. (Fixed and stained as noted.)

Fig. 11. A uninucleate cyst. Four chromatoid bodies are present and the nucleus is probably in a very early anaphase. (Fixed and stained as noted.)

Figs. 13 and 14. Mature quadrinucleate cysts, all of which contain chromatoid bodies. (Fixed and stained as noted.)